carefully planned for us, and visits to laboratories alternated with visits to the many and well-known attractions of the city. I gave a lecture and took part in a discussion on the prospects of human genetics.

The Institute of Medical Genetics is housed in a four-story building, about half of which is shared with the Endocrinology Institute. It is subdivided into the following departments:

Molecular Biology and Genetics: three laboratories—molecular biology, molecular genetics, and biochemical genetics—headed, respectively, by Drs. D. M. Spitkowsky, V. N. Kalinin, and S. S. Shishkin;

Cytogenetics: three laboratories—general genetics (Dr. N. A. Ljapunova), clinical cytogenetics (Dr. N. P. Kuleshov), and cell genetics;

Phenogenetics: two laboratories—Immunogenetics (L. A. Pevnicki) and genetics of development (Prof. V. I. Ivanov);

Population Genetics: four laboratories—mutagenesis, (Prof. N. P. Bochkov), ecogenetics (Dr. V. A. Spitsyn), population genetics (Prof. E. K. Ginter), and mathematical methods (Dr. A. N. Chebotarev);

Prevention of Hereditary Diseases: three laboratories—clinical genetics, prenatal diagnosis (Dr. A. M. Kuliev), and monitoring of biochemical defects (Dr. K. D. Krasnopolskaya).

There is also a Center of Genetic Counselling and a Department of Scientific Service. The latter includes the library, an information service, and a group for international relations.

Thus, the activities of the institute span the prevention of hereditary diseases, including genetic counseling and prenatal diagnosis, with special interest for phenylketonuria, collagenoses, cystic fibrosis, and other diseases; the monitoring of occupational health hazards and relations with congenital malformations, abortion, and chromosome aberrations; the geography of hereditary disease in the USSR; and "ecological genetics," or the study of genetic polymorphisms with attention to special groups and to living and working environments. Methods of study include two-dimensional analysis of proteins, RFLPs, and cytogenetic polymorphisms. There is a cytogenetic register. There are also other lines of research which I have not tried to cover exhaustively.

Currently there exist collaborations with several scientific centers in France, Hungary, Czechoslovakia, Poland, and several other countries. Every year, five to seven leading specialists are invited for lectures, consulting, and exchange of information. The Institute of Medical Genetics has a bank of information on activities in human genetics in the USSR. I also found of considerable interest a visit to the Laboratory of Human Population Genetics and the Laboratory of Genetic Epidemiology, both of the Institute of General Genetics (N. Vavilov) of Moscow University. This facility is located about 25–30 min by car from the Institute of Medical Genetics.

The Institute of Medical Genetics has expressed the desire to extend exchanges of scientists. Here at Stanford we currently have, as guest research worker, Dr. Nina Titenko from the Laboratory of Ecogenetics, led by Dr. Spitsyn. She is practicing techniques of study of polymerase chain-reaction (PCR) polymorphisms on some unique Russian and Siberian populations, the DNA of which she brought with her. We provide her living expenses and fund her travel within the United States. There was no problem with obtaining a visa for her. In our experience, one should count on a total wait of 5 or 6 wk and a phone call from the State Department. I hope American colleagues will be interested in establishing similar exchanges.

The postal address is Institute of Medical Genetics, USSR Academy of Medical Sciences, Moskvorechie Street 1, 115478 Moscow, USSR. The Institute is 20 years old and has about 80 Ph.D.'s. It will soon be reorganized into an All Union Center of Medical Genetics, almost tripling its current size.

L. Luca Cavalli-Sforza

Genetics Department
Stanford Medical School

Am. J. Hum. Genet. 45:337–339, 1989

A PCR Artifact: Generation of Heteroduplexes

To the Editor:

The polymerase chain reaction (PCR) (Saiki et al. 1988) is a powerful tool for diagnosing genetic diseases. We report a PCR artifact, the formation of heteroduplexes from the amplified products of homologous loci, that may prove useful in the identification of heterozygotes carrying deletion/insertion mutations. The phenomenon was identified during studies of mYfin, the candidate sequence for the mouse testis-determining Y gene (*Tdy*) (Nagamine et al. 1989). Two homologous sequences of mYfin exist on the mouse Y: *Zinc-finger y-1*

and *Zfy-2* (Nagamine et al. 1989). Primers flanking a 600-bp segment of mYfin were used to analyze (*a*) normal and sex-reversed (*Sxr*) XXSxr male mice, both of which possess *Zfy-1* and *Zfy-2* loci, and (*b*) XXSxr′ males, an *Sxr* variant possessing only *Zfy-1*. Discrepant results were obtained when the amplified samples were resolved on agarose gels versus polyacrylamide gels. In agarose gels, only two bands, correlating with *Zfy-1* and *Zfy-2*, were obseved (fig. 1*a*). In contrast, polyacrylamide gels resolved two additional fragments (A and B) in normal and XXSxr male samples (fig. 1*b*). In agarose gels the A and B fragments in normal and XXSxr male samples probably comigrate with *Zfy-1*, since this band stains more intensely (fig. 1*a*). Further investigation suggested that A and B were heteroduplexes composed of one *Zfy-1* and one *Zfy-2* strand. First, A and B were only observed when both *Zfy* loci were present. Second, when the amplified products were resolved on 6% polyacrylamide–50% urea denaturing gels, only *Zfy-1* and *Zfy-2* single strands were detected (Nagamine et al., submitted). Third, A and B could be created by combining XXSxr′ (*Zfy-1*) and mYfin (*Zfy-2*) amplified products, both of which lack A and B (fig. 1*c*). The spurious migration of A and B in polyacrylamide gels was hypothesized to be due to secondary structure (Wu and Crothers 1984) and to differences in nucleotide composition. The region responsible for the anomalous migration of A and B was localized, by restriction-enzyme digestion, to the first 126 bp (data not shown). Sequencing data identified an 18-bp deletion in *Zfy-2* at position 102 (Nagamine et al., submitted). We propose that the secondary structure in the A and B heteroduplexes is due to the *Zfy-1* and *Zfy-2* strands being annealed along their lengths, leaving the 18 extra nucelotides in the *Zfy-1* strand to form a bulge. Alternatively, the two strands may be annealed only 3′ of the deletion, leaving the 5′ ends separated. In any case, the secondary structure that is generated is responsible for the anomalous migration of A and B relative to *Zfy-1* and *Zfy-2*. Southern blot hybridization with a sense 26-mer oligonucleotide that included the 18-bp deletion and is thus specific for *Zfy-1* was performed to further categorize A and B. This oligonucleotide recognized only the *Zfy-1* and B fragments (data not shown). This suggests that the spurious migration of A and B relative to each other is due to differences in nucleotide composition—i.e., to A and B being composed of complementary *Zfy-1* and *Zfy-2* strands—and is not due to differences in secondary structure, since, in the latter case, both A and B fragments would have been recognized. The generation of heteroduplexes will theoretically occur in any PCR reaction in which two or more homologous genes or alleles are amplified using the same primers. Denaturing gradient gel electrophoresis studies have long exploited heteroduplexes to identify single-base mutations (Sheffield et al. 1989). Our data demonstrate that, if deletions/insertions in a gene being diagnosed by PCR are sufficiently large, heterozygote carriers may be identified by analyzing the amplified products on native polyacrylamide gels.

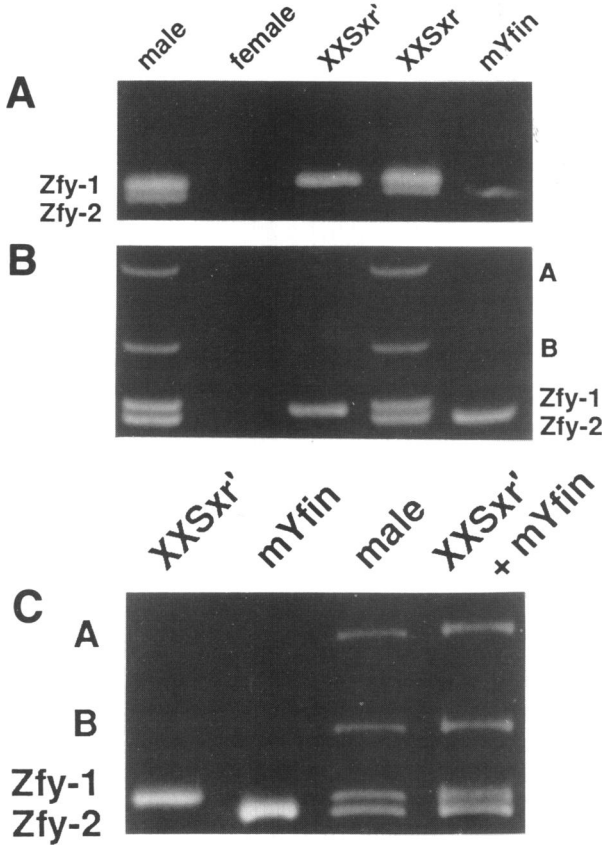

Figure 1 PCR-amplified products when DNAs derived from normal male and female mice, XXSxr′ and XXSxr sex-reversed mice, and pUC18 containing mYfin are used. The samples were resolved using (*a*) a 2% NuSieve GTG (FMC BioProducts) agarose gel and (*b*) a 10-cm 6% polyacrylamide gel, both in TBE buffer. A and B = heteroduplexes. The PCR protocol was previously described (Nagamine et al., 1989). (*c*) Synthesis of A and B from XXSxr′ (*Zfy-1*) and mYfin (*Zfy-2*) amplified products. Isolated *Zfy-1* and *Zfy-2* fragments were mixed, denatured in 50% formamide at 70°C, allowed to renature at room temperature, and then resolved on a 6% polyacrylamide gel. Lane 3, Untreated normal male.

Claude M. Nagamine, Kaimin Chan, and Yun-Fai Chris Lau
Howard Hughes Medical Institute and Departments of Physiology and Medicine University of California, San Francisco

Acknowledgments

We thank B. Elder, D. Lin, and T. Suzuki for helpful discussions and especially Y. W. Kan, who suggested the heteroduplex hypothesis. This research was funded in part by HHMI and NIH.

References

Nagamine CM, Chan K, Kozak CA, Lau YF (1989) Chromosome mapping and expression of a putative testis-determining gene in mouse. Science 243:80–83

Nagamine CM, Chan K, Lau YFC. Expression of the candidate testis-determining gene (*Zfy*) is linked to spermatogenesis in the mouse. (submitted)

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sheffield VC, Cox DR, Lerman LS, Myers RM (1989) Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. Proc Natl Acad Sci USA 86:232–236

Wu H, Crothers DM (1984) The locus of sequence-directed and protein-induced DNA bending. Nature 308:509–513